19

Mitochondrial DNA D-loop variation and implications for stock structure of the four-wing flyingfish, *Hirundichthys affinis*, in the central western Atlantic

CHARMAINE GOMES, HAZEL A. OXENFORD AND RICHARD. B. G. DALES

Correct citation: Gomes, C., H.A. Oxenford and R.B.G. Dales. 1999. Mitochondrial DNA D-loop variation and implications for stock structure of the four-wing flyingfish, *Hirundichthys affinis*, in the central western Atlantic. Bulletin of Marine Science 64: 485-500

BULLETIN OF MARINE SCIENCE, 64(3): 485–500, 1999

MITOCHONDRIAL DNA D-LOOP VARIATION AND IMPLICATIONS FOR STOCK STRUCTURE OF THE FOUR-WING FLYINGFISH, *HIRUNDICHTHYS AFFINIS,* IN THE CENTRAL WESTERN ATLANTIC

C. Gomes, H. A. Oxenford and R. B. G. Dales

ABSTRACT

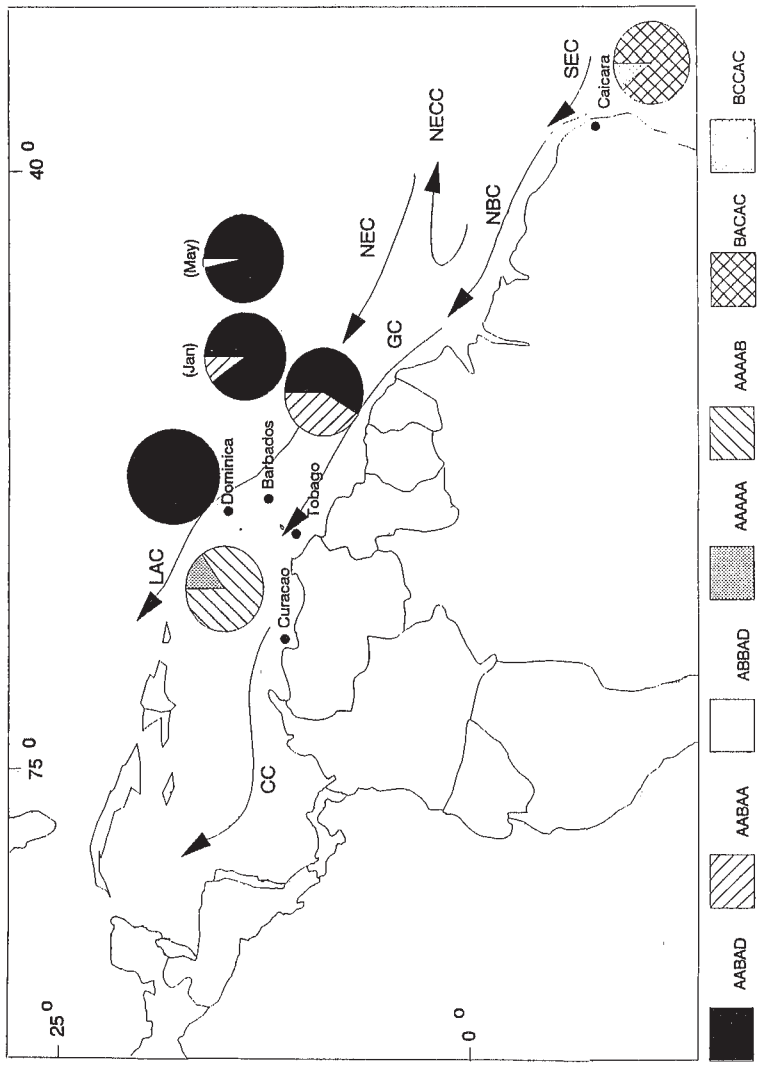
The four-wing flyingfish, *Hirundichthys affinis,* is an epipelagic, open-water species found throughout the tropical (central) Atlantic. In the central western Atlantic it supports commercially important fisheries in three geographically separate areas: the eastern Caribbean islands; the southern Netherlands Antilles; and northeast Brazil, although the resource remains unmanaged in all three areas and the stock structure unresolved. Previous tagging studies of *H. affinis,* in the central western Atlantic indicated very little about the movements of flyingfish off northeastern Brazil, but did show that flyingfish move freely between the eastern Caribbean islands, although they do not appear to travel as far as the southern Netherlands Antilles. However, tagging studies only address geographical movement of fish and cannot discriminate among genetically discrete stocks. In this study, we use mtDNA markers (restriction fragment length polymorphisms [RFLPs] of the D-loop region) to examine the genetic variation and its implication for the stock structure of flyingfish across the central western Atlantic. A total of 360 flyingfish were sampled from the commercial fisheries in the three geographical areas between January and August 1995. Sixty fish were taken from each of the two spawning populations in Barbados and 60 from the spawning populations in each of Dominica and Tobago (in the eastern Caribbean); 60 from Curaçao (in the southern Netherlands Antilles); and 60 from Caiçara (in Rio Grande do Norte, northeast Brazil). The mtDNA D-loop region was amplified using the polymerase chain reaction (PCR) method and digested to produce RFLPs using five restriction enzymes. Extensive genetic diversity was observed, with distinct composite mitotypes being detected for each of the three geographical areas, indicating a lack of gene flow between these areas and the existence of at least three unit stocks of *H. affinis* in the central western Atlantic. The results of cluster analyses of composite mitotype sequence divergence and population sequence divergence, and parsimony analysis of composite mitotypes were entirely consistent with a 3-stock model. Furthermore, genetic heterogeneity was detected among eastern Caribbean populations indicating restricted gene flow even within a sub-region and emphasizing the need for more detailed studies of flyingfish spawning behaviour. These results contrast with the typically low levels of genetic variation reported for oceanic pelagic species, and for other marine species (reef fish, spiny lobster, queen conch) in the Caribbean and indicate that major ocean current patterns are not good predictors of gene flow for all species. The implications for management of the flyingfish resource in the central western Atlantic are that three independent stock assessments and management strategies would be appropriate; and that management of the eastern Caribbean stock will need to be at a regional level since the stock is shared between the different island states, while management of the southern Netherlands Antilles stock and the northeast Brazil stock could be at a national level.

Fish species tend towards genetically discrete populations (unit stocks) that are essentially self-sustaining and react to harvesting, more or less, independently (Carvalho and Hauser, 1995). Information on the genetic stock structure of fish populations is therefore fundamental to fisheries management, especially for commercially important fish species whose geographic distributions transcend national boundaries, to determine whether management should be nation or region-specific (Ferris and Berg, 1987).

The four-wing flyingfish, *Hirundichthys affinis,* is distributed throughout the

epipelagic zone of the tropical Atlantic, with concentrations of abundance in the central western Atlantic occurring in the Caribbean Sea, Gulf of Mexico and off the northeast coast of Brazil (Brunn, 1935; Breder, 1938; Fischer, 1978; Oxenford et al., 1995). It is a commercially valuable species throughout the eastern Caribbean, where it represents the first or second most important species landed by most of the islands (Mahon et al., 1986; Oxenford et al., 1993), in the southern Netherlands Antilles (Zaneveld, 1961; G. Van Buurt, Director, Fishery Sector, Department of Agriculture, Animal Husbandry and Fisheries, Klein Kwartier, Curaçao, pers. comm.) and off the northeast coast of Brazil (Da Cruz, 1965; Monteiro et al., 1996). Very little is known about the stock structure of *H. affinis* and no form of resource management is currently practised for this species by any of the countries harvesting flyingfish. This lack of knowledge is of particular concern to countries of the eastern Caribbean, where pelagic fleet sizes are continuing to expand (Oxenford, 1991; Lawrence, 1993; Samlalsingh et al., 1993; Willoughby, 1993) and serious efforts are now being made to conduct resource assessments and to draw up rational fishery management plans (CFRAMP, 1996a,b).

Tagging studies to examine local and regional movements of *H. affinis* have been conducted off Barbados (Mulloney, 1961; Lewis, 1964) and the eastern Caribbean (Oxenford, 1994) and off the northeast coast of Brazil (Barroso, 1967). The Barbados studies indicated that at least some flyingfish remain in, or return to, the release location over a period of up to 50 d (Mulloney, 1961; Lewis, 1964). The eastern Caribbean study demonstrated that although *H. affinis* may remain in the release location for several weeks, they do move freely between the islands, suggesting that eastern Caribbean nations share a common unit stock (Oxenford, 1994). The Brazil study was inconclusive since recapture rates were low and mean time at large was only three days, suggesting that the fish had not migrated from the point of release (Barroso, 1967). However, tagging studies only address geographical movement of fish and cannot determine whether fish populations that appear to be mixing, are actually interbreeding, nor can they discriminate among genetically discrete stocks (Milner et al., 1985; Hynes et al., 1989; Graves and McDowell, 1994). It remains possible then, that despite detected mixing of adult *H. affinis* in the eastern Caribbean, there may be a more complex stock structure in this region if adults are segregating to specific areas for spawning (Oxenford, 1994), and early life history stages are being retained by localised current gyres (Kinder, 1983). Furthermore, two peaks in spawning activity of *H. affinis* have been detected off Barbados (Storey, 1983; Khokiattiwong, 1988; Lao, 1989), a minor one in December/January and a major one in April/May. This may indicate the presence of two genetically distinct, but sympatric, stocks of flyingfish, each with its own spawning period. If this is so, then management options will need to consider protection of both stocks (Oxenford et al., 1993). Alternatively, since *H. affinis* are believed to be, for the most part, pelagic spawners with an extended spawning season (Oxenford, 1986; Hunte et al., 1995) there is the potential for shared recruitment over a wide area of the Caribbean via passive transport of early life history stages by the westward flowing Caribbean Current and the northwesterly flowing Lesser Antilles Current (Froelich et al., 1978; Kjerfve, 1986; Gable, 1993; Fig. 1) such that populations in the eastern Caribbean, the southern Netherlands Antilles and elsewhere downstream, may constitute a single stock. The Caribbean and northeast Brazil populations are less likely to share adults or recruits, since the two areas are some 3500 km apart. However, water masses from northeast Brazil are known to reach the Caribbean seasonally (Muller-Karger et al., 1988, 1989; Petersen and Stramma, 1991; Didden and Schott, 1993; Fran-



NBC-North Brazil Current CC-Caribbean Current GC-Guiana Current NEC-North Equatorial Current
NECC-North Equatorial Counter Current LAC-Lesser Antilles Current SEC-South Equatorial Current

Figure 1. Map of the central western Atlantic showing location of sampling sites; approximate position of major surface water currents; and geographic distribution and percent frequency of composite mitotypes of flyingfish, *Hirundichthys affinis*.

Table 1. Sampling locations, dates and sample sizes for spawning flyingfish (*H. affinis*) from the central western Atlantic.

Geographical area	Country	Landing site	Date	No. of fish
Eastern Caribbean	Barbados	Bridgetown	January (3rd–24th)	20
		Conset Bay		20
		Half Moon Fort		20
		Bridgetown	May (1st–22nd)	20
		Conset Bay		20
		Half Moon Fort		20
	Dominica	Marigot	April (3rd–7th)	29
		Newtown		9
		Sainte Sauveur		22
	Tobago	Buccoo	April (14th–17th)	60
Southern Netherlands Antilles	Curaçao	Boca Simon	July (25th–28th)	1
		Caracasbaai		1
		Watamul		58
Brazil	Brazil	Caiçara	August (25th–31st)	60

tatoni et al., 1995; Fig. 1) so that flyingfish throughout the central western Atlantic may comprise a single stock.

Given the constraints of tagging studies, it is prudent to examine stock structure at the genetic level, using molecular biology techniques (Milner et al., 1985). Molecular biology techniques have become increasingly important for determining fish stock structure (Carvalho and Pitcher, 1995). In particular, analysis of restriction-site polymorphisms of mitochondrial DNA (mtDNA), which because of its maternal mode of inheritance and rapid evolution (Birky et al., 1983; Hynes et al., 1989; Brown et al., 1992) has become a widely-used population genetics tool in fisheries (Ovenden, 1990; Zwanenburg et al., 1992; Camper et al., 1993; Crosetti et al., 1994; Kotoulas et al., 1995). In this study we use mtDNA D-loop markers to investigate the stock structure of *H. affinis* in the central western Atlantic for the first time, and address the implications for management of the resource. Selection of the mtDNA D-loop for analysis was based on its documented hypervariability in many fish species (Ovenden, 1990; Cronin et al., 1993; Alvarado Bremer, 1994) and the availability of specific primers for amplification of this region (Palumbi et al., 1991). Specifically, we examine the genetic variation between Caribbean and Brazil populations, and further test the hypothesis of a single Caribbean stock (shared between the eastern Caribbean islands and the southern Netherlands Antilles).

Materials and Methods

Sample Collection and Preservation.—A total of 360 running ripe (spawning) *H. affinis* were sampled from commercial fishermen in three distinct geographical areas within the central western Atlantic (eastern Caribbean, southern Netherlands Antilles and Brazil) in 1995, during the periods of peak flyingfish spawning activity in each area. Within the eastern Caribbean, samples were taken from three widely spaced countries: Barbados in January (n = 60) and May (n = 60), Dominica in April (n = 60) and Tobago in April (n = 60). Within the southern Netherlands Antilles samples were taken from Curaçao in July (n = 60), and within Brazil samples were taken from Caiçara on the northeast coast, in August (n = 60; Table 1; Fig. 1). These sampled populations are referred to as Barbados (January), Barbados (May), Dominica, Tobago, Curaçao and Brazil. Wherever possible samples were taken from several different boats over a period of days and from different landing sites, to minimize the possibility of only sampling from a single school of fish.

Liver tissue (selected for its high DNA yield and easy homogenization) was removed from all

freshly landed fish (which had been kept on ice up to a maximum of 7 h), and placed in preservative buffer (20% [v/v] DMSO and 0.25 M Na_2EDTA saturated with 1 M NaCl).

DNA EXTRACTION.—Genomic DNA was isolated from the liver tissue following a slightly modified version of the protocol described by Cheung et al. (1993). For each sample approximately 5 mg of liver tissue was washed in 20 µl 10 % (w/v) CTAB detergent to remove mucous and homogenised with a pestle in extraction buffer (200 mM Tris-HCl [pH 8.0], 70 mM EDTA [pH 8.0], 2 M NaCl, 20 mM sodium metabisulphite). Cell membranes were disrupted by the addition of 40 µl 5% (w/v) Sarkosyl and the lysate incubated at 60°C for 1 h and centrifuged for about 15 min at 1300 xg to separate the DNA (clear aqueous phase) from the cellular debris. The DNA in the supernatant was then precipitated by the addition of 90 µl 10 M ammonium acetate and 200 µl isopropyl alcohol and centrifuged into pellet form. The DNA pellet was rinsed with 17 µl 70% ethanol, air-dried, resuspended in 150 µl TE buffer (10 mM Tris [pH 8.0], 1 mM EDTA [pH 8.0]) containing 10 µg ml^{-1} RNase and stored at −20°C.

AMPLIFICATION OF MITOCHONDRIAL D-LOOP REGION.—The D-loop region of mtDNA was amplified from the genomic DNA using the polymerase chain reaction (PCR) technique and two primers (5′—CTACCTCCAACTCCCAAAGC − 3′ and 5′—CCTGAAGTAGGACCAGATG − 3′; Operon Technologies Incorporated) designed to target the tRNA genes flanking the D-loop region (Palumbi et al., 1991).

PCR was carried out in a final volume of 25 µl, in a reaction mix described by Kocher et al. (1989). This reaction mix was preheated at 94°C for 4 min followed by 40 cycles of amplification (94°C for 1 min, 55°C for 1 min and 72°C for 2 min). The reaction mix was subjected to a final step at 72°C for 10 min.

ENDONUCLEASE DIGESTION OF AMPLIFIED MTDNA.—Eleven restriction endonucleases (*Alu*I, *Dpn*II, *Hae*III, *Hha*I, *Hin*P1I, *Hpa*I, *Mbo*I, *Mse*I, *Nla*III, *Rsa*I and *Taq*I; New England Biolabs), recognizing tetranucleotide sequences were screened using a sub-sample of twelve flyingfish for their ability to cut the D-loop amplicon. Of these, five (*Hha*I, *Hin*P1I, *Mbo*I, *Mse*I, *Rsa*I) were found to be informative, producing variable banding patterns.

One unit of each enzyme and its specific buffer were applied to 6 µl amplified mtDNA product and the reactants were incubated at a temperature appropriate for the particular enzyme. The digested mtDNA samples were separated through 2% agarose (Sigma Chemical Company) gels in TAE buffer (40 mM Tris-acetate, 1 mM EDTA) by electrophoresis at 70 v s^{-1} for approximately 4 h. Gels were stained with ethidium bromide, viewed under ultra violet light to visualise restriction fragment patterns (mitotypes), and photographed.

DATA ANALYSIS.—Following electrophoresis, the sizes of the amplified D-loop region and the digested fragments were estimated by semi-log plot comparisons of distance travelled by each fragment with distance travelled by known size fragments of molecular weight markers (lambda DNA digested with *Eco*RI and *Hin*dIII [Sigma Chemical Co.] and pUC 19 digested with *Dde* I).

Mitotypes generated by each endonuclease were designated by a capital letter in order of detection. Composite mitotypes were constructed from five letters corresponding to the mitotypes of *Hha*1, *Hin*P1I, *Mbo*I, *Mse*I and *Rsa*I, respectively.

Genetic heterogeneity across all sampled *H. affinis* in the central western Atlantic was investigated by examining overall mean and intra-population mean mitotype (nucleon) and nucleotide sequence diversities, and composite mitotype sequence divergences. These were computed using the REAP package (McElroy et al., 1992) following the formulations of Nei and Li (1979), Nei and Tajima (1981) and Nei (1987). Genetic relatedness of these fish was examined by cluster and parsimony analyses of composite mitotypes. Composite mitotypes were clustered by the UPGMA method based on estimates of nucleotide sequence divergence among them, using the Neighbor program in the Phylip software package (ver. 3.5c, Felsenstein, 1993). A maximum parsimony network of composite mitotypes, based on the variations in nucleotide sequences of restriction sites, was constructed. This was achieved by bootstrapping (100 randomizations) the presence/absence matrix of restriction sites, clustering (Wagner parsimony) the resulting matrices and generating a single, strict consensus tree using the Seqboot, Mix and Consense programmes in the Phylip software package (ver. 3.5c, Felsenstein, 1993).

The extent of genetic heterogeneity among sampled populations was investigated by comparing mitotype frequencies and examining inter-population nucleotide sequence divergences. Composite mitotype frequencies were compared among populations through a Monte Carlo simulation (Roff and Bentzen, 1989), using the MONTE programme in REAP and 1000 bootstrapped replicates. This procedure avoids problems created by the occurrence of many cells with low expected frequencies, such as occurs with rare mitotypes and small sample sizes. Inter-population nucleotide sequence divergences were computed and clustered using the REAP and Phylip software packages respectively, as explained above.

RESULTS

AMPLIFICATION AND DIGESTION OF D-LOOP.—The primers consistently amplified a 480 bp DNA fragment of the mitochondrial D-loop in all individuals, without

Table 2. Mitotypes and estimated fragment sizes produced by restriction endonuclease digestion of the mitochondrial D-loop region of *H. affinis* samples from the central western Atlantic.

Restriction endonuclease	Mitotype	Fragment sizes (bp)
*Hha*1	A	480
	B	445, 35
*HinPI*1	A	480
	B	420, 60
	C	310, 170
*Mbo*1	A	480
	B	340, 140
	C	310, 170
*Mse*1	A	325, 155
*Rsa*1	A	480
	B	290, 190
	C	250, 230
	D	245, 235

apparent size differences among or within individuals from different sampled populations.

Restriction endonuclease *Hha*1 produced two mitotypes (A and B), *Hin*P1I and *Mbo*I each produced three mitotypes (A, B and C), *Mse*I produced a single mitotype (A) and *Rsa*I produced four mitotypes (A, B, C and D) giving a total of seven composite mitotypes across the total sample size of 360 flyingfish (Table 2; Figure 2).

GENETIC HETEROGENEITY ACROSS ALL FISH.—Over the entire *H. affinis* survey, the probability that randomly chosen pairs of individuals in the central western Atlantic would have a different composite mitotype was 0.198, and the mean nucleotide sequence diversity between any pair was 0.048 (Table 3).

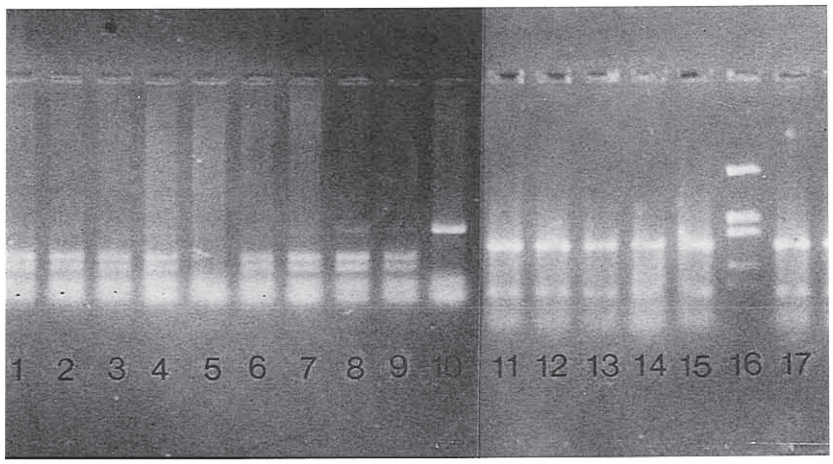


Figure 2. Photograph of a gel showing band patterns of PCR products and restriction digest products in flyingfish (*H. affinis*). Lanes 1–9 represent fragment patterns (mitotype C) detected in flyingfish from Curaçao digested with *Rsa*I; lane 10 is the PCR-amplified mtDNA D-loop region; lanes 11–15 and lane 17 represent fragment patterns (mitotype C) detected in flyingfish from Tobago digested with *Mbo*I; and lane 16 is the molecular marker pUC19 cut with *Dde*I.

Table 3. Genetic heterogeneity of the mtDNA D-loop region of *H. affinis* from six sampled populations within the central western Atlantic, expressed as mean intra-population mitotype diversity (h) and mean intra-population nucleotide sequence diversity (π).

Geographical location	Sampled populations	No. of individuals	Mitotype diversity index (h)	Nucleotide diversity (π)
Eastern Caribbean	Dominica	60	0.000	0.0000
	Barbados (Jan)	60	0.183	0.0035
	Barbados (May)	60	0.066	0.0011
	Tobago	60	0.494	0.0094
Southern Netherlands Antilles	Curaçao	60	0.259	0.0058
Brazil	Brazil	60	0.183	0.0027
Pooled sample mean (SE)		360	0.198 ± (0.005)	0.048 ± (0.001)

All sampled populations, with the exception of Dominica, exhibited multiple composite mitotypes with intra-population mitotype diversity (h) ranging from 0 in Dominica to 0.494 in Tobago (Table 3). Mean intra-population nucleotide sequence diversity (π) ranged from 0 in Dominica to 0.0094 in Tobago (Table 3).

Estimates of nucleotide sequence divergence (p) among the seven composite mitotypes (Table 4) indicated that levels of nucleotide sequence divergence between individuals ranged from 1.45% to 14.4%. Cluster analysis resolved the seven composite mitotypes into three major groups which align perfectly with the three geographical areas studied in the central western Atlantic, i.e., the eastern Caribbean islands, the southern Netherlands Antilles and Brazil (Fig. 3). The level of mitotype sequence divergence between individuals from the eastern Caribbean and southern Netherlands Antilles was 2.6%, and between Caribbean populations and Brazil was 5.7% (Fig. 3).

Maximum parsimony analysis of the restriction-site presence/absence matrix for individual mitotypes also resolved the composite mitotypes into three groups corresponding exactly to their geographical locations (Fig. 4). The most common mitotype of Brazil differed from that of the eastern Caribbean by gaining three restriction sites for the enzymes *Hha*I, *Mbo*I and *Rsa*I and by losing two restriction sites for the enzymes *Mbo*I and *Rsa*I. Within the Caribbean, the most common mitotype of the southern Netherlands Antilles differed from that of the eastern Caribbean by losing two restriction sites for the enzymes *Mbo*I and *Rsa*I, and gaining a site for *Rsa*I (Fig. 4; Table 2).

INTER-POPULATION GENETIC HETEROGENEITY.—Composite mitotypes AABAD,

Table 4. Genetic heterogeneity of the mtDNA D-loop region of *H. affinis* from six sampled populations within the central western Atlantic, expressed as estimated percent nucleotide sequence divergence (p) between mitotypes.

	AABAD	ABBAD	AABAA	AAAAA	AAAAB	BACAC	BCCAC
AABAD	—						
ABBAD	1.893	—					
AABAA	1.644	4.062	—				
AAAAA	4.868	2.232	8.162	—			
AAAAB	5.494	4.868	8.772	2.232	—		
BACAC	9.350	8.772	13.864	8.162	8.772	—	
BCCAC	13.864	13.296	14.404	12.697	13.296	1.452	—

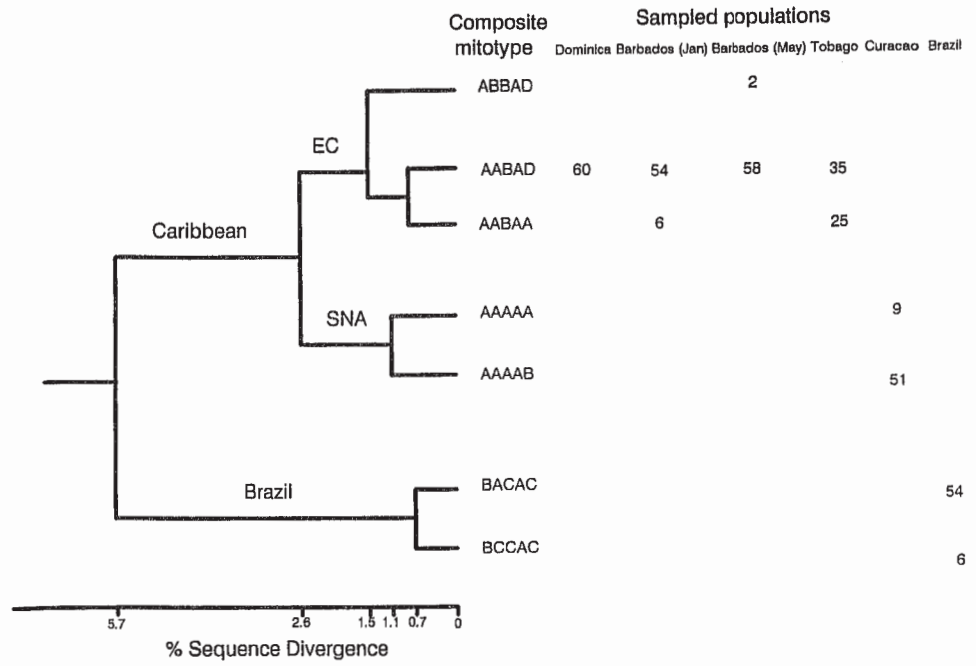


Figure 3. UPGMA phenogram showing geographical distribution and genetic relatedness of composite mitotypes observed in 360 *H. affinis* from the central western Atlantic. Letters represent mitotypes produced by *Hha*I, *Hin*P1I, *Mbo*I, *Mse*I and *Rsa*I, respectively. EC- eastern Caribbean, SNA-Southern Netherlands Antilles.

BACAC and AAAAB were the most common, occurring in 57.5%, 15.0% and 14.2% of individuals, respectively (Figs. 1,3). Interestingly, no composite mitotypes were shared among the three geographical areas. However, mitotypes were shared between sampled populations within the eastern Caribbean (Figs. 1,3), although they occurred with significantly different frequencies in the different populations (Monte Carlo Chi squared 4×3 contingency test; max. $X^2_r = 21.58 < X^2_o = 67.88$, $P < 0.001$). Further analysis of genetic heterogeneity within the eastern Caribbean populations, excluding each of the populations in turn, revealed significant heterogeneity among all populations (max. $X^2_r < X^2_o$, $P \leq 0.001$ in all cases).

Estimates of nucleotide sequence divergence among sampled populations ranged from 0.001% to 9.75% (Table 5). Cluster analysis resolved the six populations into three groups aligning perfectly with the three geographical areas (Fig. 5). Within the eastern Caribbean, levels of sequence divergence between populations was less than 0.14%. Within the Caribbean, eastern Caribbean populations showed a sequence divergence of 2.42% from the southern Netherlands Antilles population, and the Caribbean populations showed a sequence divergence of 4.64% from the Brazil population (Fig. 5).

Discussion

Estimation of genetic variation within conspecific fish populations has been enhanced by the recent, but still preliminary, development of statistical techniques for evaluating population-level mtDNA data. We therefore utilized a variety of techniques here to determine the presence and extent of genetic differentiation among geographically separated populations of flyingfish.

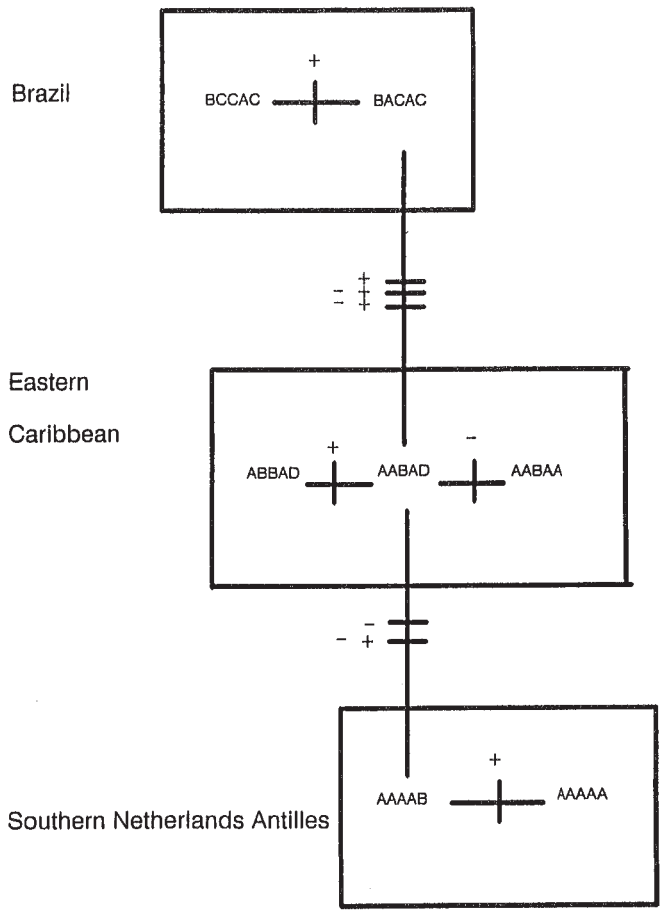


Figure 4. Parsimony network showing genetic relatedness of composite mitotypes observed in 360 *H. affinis* from the central western Atlantic. Letters represent mitotypes produced by *Hha*I, *Hin*P1I, *Mbo*I, *Mse*I and *Rsa*I, respectively. + indicates the addition of a restriction site, − indicates the loss of a restriction site.

Table 5. Genetic heterogeneity of the mtDNA D-loop region of *H. affinis* from six sampled populations within the central western Atlantic, expressed as percent inter-population nucleotide sequence divergence (p).

Country	Dominica	Barbados (Jan)	Barbados (May)	Tobago	Curaçao	Brazil
Dominica	—					
Barbados (Jan)	0.016	—				
Barbados (May)	0.001	0.019	—			
Tobago	0.321	0.179	0.329	—		
Curaçao	5.111	4.845	5.166	4.256	—	
Brazil	9.668	9.437	9.751	8.960	8.711	—

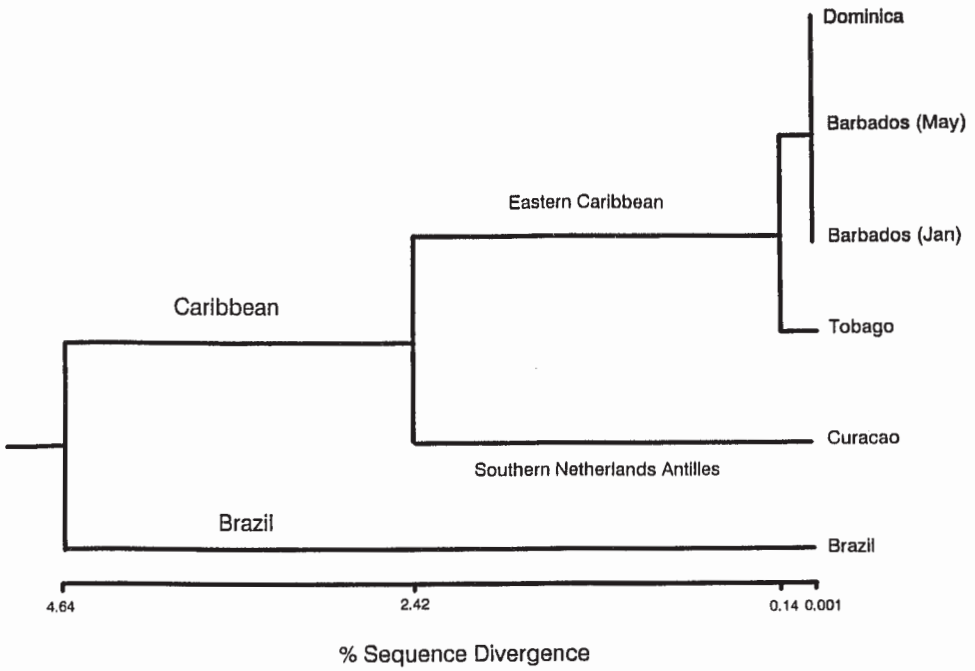


Figure 5. UPGMA phenogram showing geographical distribution and genetic relatedness of six sampled populations of *H. affinis* from the central western Atlantic.

The presence of distinct (diagnostic) composite mitotypes in each of the three geographical areas (eastern Caribbean, southern Netherlands Antilles and northeast Brazil; Fig. 1) provides conclusive evidence of a lack of material gene flow (at present or in the recent past) among the sampled populations from these three locations (Crow, 1986; Ovenden, 1990), and indicates the existence of at least three unit stocks of flyingfish in the central western Atlantic. Cluster analysis of composite mitotype sequence divergence estimates (1.5–14%; Table 4) grouped them into three cohesive units aligning perfectly with the three geographical areas (Fig. 3). This supports the conclusion that there are at least three unit stocks of flyingfish in the central western Atlantic. This analysis further indicated that sampled populations within the Caribbean (i.e., from the eastern Caribbean and the southern Netherlands Antilles) are more closely related to each other than to the sampled population from Brazil, although the percent sequence divergences are of the same order of magnitude (2.6% divergence between eastern Caribbean and southern Netherlands Antilles mitotypes; 5.7% divergence between Caribbean and Brazil mitotypes; Fig. 3). Maximum parsimony analysis of composite mitotypes further supported the 3-stock model and again indicated greater genetic separation between eastern Caribbean and Brazil mitotypes (5 restriction site changes) than between eastern Caribbean and southern Netherlands Antilles mitotypes (3 restriction site changes; Fig. 4). Lastly, cluster analysis of sequence divergence among populations also produced three groups aligning with geographic origin, and indicating greater separation between Caribbean and Brazil populations (4.6% divergence), than between eastern Caribbean and southern Netherlands Antilles populations (2.4% divergence; Fig. 5). The results of all the analyses are consistent with one another and do not support the suggestion of a simple stock structure

(i.e., a single panmictic population) for flyingfish within the central western Atlantic, nor the hypothesis of a single Caribbean stock.

In general, the dispersal abilities of organisms and the presence or absence of geologic and/or oceanographic barriers to dispersal will determine the degree of gene flow between spatially separated populations. Disjunct geographical distribution provides the opportunity for the evolution of genetically distinct populations (unit stocks). However, if the barriers isolating populations vary or break down temporarily, and the distances separating them are not too large to prevent dispersal, then a lack of significant genetic heterogeneity would be expected (Shulman and Bermingham, 1995). Oceanic pelagic fish, for example flyingfish, which have pelagic eggs, larvae and adults can be expected to have high dispersal abilities, through migration of adults and passive concurrent dispersal of eggs and larvae by surface water currents, and hence low genetic variation over broad areas. For example, very low levels of genetic variation have been reported for oceanic pelagic species such as skipjack tuna (Graves et al., 1984), albacore (Graves and Dizon, 1989), armorhead (Martin et al., 1992), Spanish sardine (Tringali and Wilson, 1993), swordfish (Alvarado Bremer et al., 1994), billfish (Graves and McDowell, 1994) and yellowfin tuna (Ward et al., 1994).

The pronounced population genetic structure found for flyingfish in this study was not anticipated, particularly given that other studies within the Caribbean have reported high rates of gene flow even in benthic species (e.g., reef fish, queen conch, spiny lobster) which have benthic or pelagic eggs and pelagic larvae (Mitton et al., 1989; Lacson, 1992; Hateley and Sleeter, 1993; Shulman and Bermingham, 1995). These studies concluded that surface currents within the Caribbean are allowing passive dispersal of pelagic eggs and larvae across broad areas of the region and have not acted as barriers to gene flow through evolutionary time (Shulman and Bermingham, 1995). Note however that a high degree of genetic variation is not unprecedented in migratory pelagic species for example sturgeon, menhaden and sea bass (Bowen and Avise, 1990); blue marlin (Finnerty and Block, 1992); grey mullet (Crosetti et al., 1994); and swordfish (Kotoulas et al., 1995).

Our results suggest that the distances between the three geographic areas (~890 km between the eastern Caribbean islands and the southern Netherlands Antilles; ~3500 km between the eastern Caribbean and northeast Brazil) are large enough to prevent migration and interbreeding of adult flyingfish among these three areas, and that surface currents are not entraining viable eggs and larvae and passively transporting them between these locations. Indeed, tagging studies of flyingfish have not detected movement of adults between these three areas (Lewis, 1964; Barroso, 1967; Oxenford, 1994). The greatest distance so far recorded for a tagged flyingfish is 370 km (between Dominica and Tobago; Oxenford, 1994).

The fact that early life history stages of flyingfish are apparently not being transported between the three locations is more surprising, given that the major ocean currents in the central western Atlantic are known to transport surface water masses from northeast Brazil to the eastern Caribbean and from the southeastern Caribbean westwards towards the southern Netherlands Antilles (Fig. 1). The major currents affecting northeast Brazil are complex and vary seasonally (see Didden and Schott, 1993; Fratantoni et al., 1995 for reviews). During the period July to January, the North Brazil Current, supplied by the South Equatorial Current, flows northwesterly along the coast from the northeastern tip of Brazil near 5°S and then separates sharply from the coast at around 6–7°N and retroflects to feed the eastward flowing North Equatorial Counter Current (e.g., Muller-Karger et al. 1988, 1989; Petersen and Stramma, 1991; Didden and Schott, 1993). As such, any flyingfish eggs and larvae in the waters off northeast Brazil are most likely to be transported eastwards

and not towards the Caribbean at this time of the year, which coincides with the reported period of peak spawning (August) for *H. affinis* in this area (Almeida, 1966; Da Cruz, 1973). This would explain the lack of gene flow between northeast Brazil and Caribbean flyingfish populations. However, spawning flyingfish do occur off northeast Brazil from as early as April/May (Almeida, 1966; Monteiro et al., 1996) when ocean currents in this area are believed to be quite different. During the period February to June it is known that the North Equatorial Counter Current is very weak or non-existent and that water masses of South Atlantic origin enter the Caribbean. The exact mechanism for this however remains in debate (Didden and Schott, 1993). It is believed that most of the North Brazil Current flows northwesterly to feed the Guiana Current which eventually enters the Caribbean through the passages between the eastern Caribbean islands. Furthermore, mesoscale eddies shedding from the North Brazil Current between October and March have also been detected, and found to propagate northwestwards, travelling to the Caribbean over a period of approximately 100 d (Johns et al., 1990; Didden and Schott, 1993; Fratantoni et al., 1995). As such, it is conceivable that early life history stages of flyingfish could be transported from northeastern Brazil to the Caribbean between February and June. This study clearly indicates that this has not been occurring and suggests that either physical conditions (such as lowered salinities in the vicinity of the Amazon outflow) are acting as a barrier to dispersal; and/or that eggs and larvae are prehaps being entrained in the south flowing Brazil Current which may be encroaching the spawning grounds during the early part of the year (see Quarterly IOC/WMO Intergrated Global Ocean Serives System [IGOSS] Products Bulletins); and/or that localised (mesoscale) currents are retaining flyingfish. Mesoscale current patterns off northeast Brazil are not well documented (Ekau and Knoppers, 1996).

Evidence for exchange of pelagic larvae between the eastern Caribbean and southern Netherlands Antilles is provided by a recent study of several benthic reef fish species which showed a lack of significant mtDNA sequence divergence between Caribbean populations (including populations from Barbados and Curaçao), and concluded that gene flow had not been constrained by present-day ocean currents (Shulman and Bermingham, 1995). However, mesoscale current features such as the many eddies and gyres which have been reported for the Caribbean (Molinari et al., 1981; Kinder, 1983) may provide an explanation. These features provide a mechanism for retention of early life history stages (Emery, 1972; Hunt von Herbing and Hunte, 1991; Cowen and Castro, 1994) and may be responsible for preventing gene flow between eastern Caribbean and southern Netherlands Antilles flyingfish populations.

Apart from the clear separation of three unit stocks of flyingfish within the central western Atlantic, we also detected small, but significant differences in composite mitotype frequencies among sampled populations within the eastern Caribbean sub-region, and sequence divergences of up to 0.14% between these populations (Fig. 5). This heterogeneity indicates at least partially restricted gene flow between the sampled populations in the eastern Caribbean, since even small rates of migration between populations should eventually homogenize any neutral genetic variation (Crow, 1986; Schultz and Cowen, 1994). Interestingly, although free movement of adults among the eastern Caribbean islands was detected with tagging, rates of adult dispersal between islands slowed considerably as fish reached full maturity, perhaps suggesting some segregation to specific spawning sites (Oxenford, 1994). If these are natal spawning sites, then restricted gene flow may be expected. Certainly, complete fidelity to natal nesting beaches has resulted in sharp genetic separation of turtle populations in the Caribbean (Bowen et al.,

1992; Bass et al., 1996). We do not consider this sub-regional heterogeneity to be indicative of further unit stock separation within the eastern Caribbean, given that the level of genetic variation detected is an order of magnitude smaller than the variation detected between flyingfish populations from the three geographical areas in the central western Atlantic (Figs. 3,5). This is consistent with the tentative conclusions of Hunte (1986) and Oxenford (1994) that there is a single shared stock of flyingfish within the eastern Caribbean sub-region. These results also reiterate the need (as outlined by Oxenford et al. (1993), Hunte et al. (1995) and Nakashima (1996)) for a greater understanding of flyingfish spawning behaviour in order to refine management and conservation of the flyingfish resource.

The existence of at least three unit stocks of flyingfish in the central western Atlantic, located in the eastern Caribbean, the southern Netherlands Antilles and northeastern Brazil, indicates that it would be prudent to conduct three independent stock assessments, and provides an appropriate geographical scale for management initiatives. Regional-level management of a single shared flyingfish stock is implicated for the eastern Caribbean states; while national-level management of a single stock would be appropriate for flyingfish in the southern Netherlands Antilles (which share a common national government), and for the flyingfish stock in northeast Brazil.

We conclude that the use of mtDNA markers is an appropriate tool for determination of genetic variation in flyingfish and thus for determination of unit stock structure and for setting an appropriate geographical scale for management efforts.

ACKNOWLEDGMENTS

Funding for this study was provided by the Marine Resource and Environmental Management Programme (MAREMP) and the Department of Biology, and by grants to C. Gomes from the Postgraduate Research Committee and to H. Oxenford from the Campus Research Fund of the University of the West Indies (U.W.I.), Cave Hill Campus, Barbados. We thank the fishermen of Dominica, Barbados, Tobago, Curaçao and Brazil for their co-operation in providing the flyingfish samples and gratefully acknowledge the field assistance of Fisheries Divisions and fishery personnel in the eastern Caribbean and southern Netherlands Antilles and of R. Lessa and her students at the University of Pernambuco in Brazil. A. Martin is thanked for his assistance in selecting appropriate primers for D-loop amplification and G. Dahle, P. Bentzen and J. Felsenstein for assistance with data analysis and interpretation.

LITERATURE CITED

Almeida, N. V. 1966. Estudos sôbre a maturidade do peixe-voador (*Hirundichthys affinis,* Günther) na costa nordestina do Brasil. B. Est. Pesca 6: 33–41.

Alvarado Bremer, J.R., J. Mejuto and B. Ely. 1994. Global population structure of the swordfish (*Xiphias gladius*), as revealed by the analysis of the mitochondrial control region. ICCAT collective volume of scientific papers vol. XLIV(13); SCRS/94/127: 206–216.

Barroso, L. M. 1967. Biologia e pesca do peixe-voador (*Hirundichthys affinis* Günther) no estado do Rio Grande Do Norte. Bol. Est. Pesca 7: 9–37.

Bass, A. L., D. A. Good, K. A. Bjorndal, J. I. Richardson, Z.-M. Hills, J. A. Horrocks and B. W. Bowen. 1996. Testing models of female reproductive migratory behaviour and population structure in the Caribbean hawksbill turtle, *Eretmochelys imbricata,* with mtDNA sequences. Mol.. Ecol. 5: 321–328.

Birky, C. W.Jr, T. Maruyama and P. Fuerst. 1983. An approach to population theory for genes in mitochondria and chloroplasts, and some other results. Genetics 103: 513–527.

Bowen, B.W. and J.C. Avise. 1990. Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: influence of zoogeographic factors and life-history patterns. Mar. Biol. 197: 371–381.

———, A. B. Meylan, J. P. Ross, C. J. Limpus, G. H. Balazs and J. C. Avise. 1992. Global population structure and natural history of the green turtle, (*Chelonis mydas*) in terms of matriarchal phylogeny. Evolution 46: 865–881.

Breder, C. M. 1938. A contribution to the life histories of Atlantic Ocean flying fishes. Bull. Bingham Oceanogr. Coll. 6: 1–126.

Brown, J. R., A. T. Beckenbach and M. J. Smith. 1992. Mitochondrial DNA length variation and heteroplasmy in populations of white sturgeon (*Acipenser transmontanus*). Genetics 132: 221–228.

Brunn, A. Fr. 1935. Flying-fishes (Exocoetidae) of the Atlantic—systematic and biological studies. Dana 6: 1–106.

Camper, J. D., R. C. Barber, L. R. Richardson and J. R. Gold. 1993. Mitochondrial DNA variation among red snapper (*Lutjanus campechanus*) from the Gulf of Mexico. Mol. Mar. Biol. Biotech. 2: 154–161.

CFRAMP. 1996a. CARICOM Fisheries resource assessment and management program (CFRAMP) final management plan. CARICOM Fisheries Unit, Belize City, Belize. 162 p.

———. 1996b. CFRAMP annual work plan 1996/1997. CARICOM Fisheries Unit, Belize City, Belize. 112 p.

Carvalho, G. R. and L. Hauser. 1995. Molecular genetics and the stock concept in fishes. Pages 53–79 *in* G. R. Carvalho and T. J. Pitcher, eds. Molecular genetics in fisheries. Chapman and Hall, London. 141 p.

——— and T. J. Pitcher, eds. 1995. Molecular genetics in fisheries. Chapman and Hall, London. 141 p.

Cheung, W. Y., N. Hubert and B. S. Landry. 1993. A simple and rapid DNA microextraction method for plant, animal, and insect suitable for RAPD and other PCR analyses. PCR Meth. Appl. 3: 69–70.

Cowen, R. and L. R. Castro. 1994. Relation of coral reef larval distributions to island scale circulation around Barbados, West Indies. Bull. Mar. Sci. 54: 228–243.

Cronin, M. A., W. J. Spearman and R. L. Wilmot. 1993. Mitochondrial DNA variation in chinook (*Oncorhynchus tshawytscha*) and chum Salmon (*O. keta*) detected by restriction enzyme anlaysis of polymerase chain reaction (PCR) products. Can. J. Fish. Aquat. Sci. 50: 708–715.

Crosetti, D., W. S. Nelson and J. C. Avise. 1994. Pronounced genetic structure of mitochondrial DNA among populations of the circumglobally distributed grey mullet (*Mugil cephalus*). J. Fish Biol. 44: 47–58.

Crow, J. F. 1986. Basic concepts in population, quantitative and evolutionary genetics. W. H. Freeman and Company, New York. 273 p.

Da Cruz, J. F. 1965. Sobre a biologia pesqueira do voador, *Hirundichthys affinis* (Günther 1866), no Nordeste do Brazil. Bol. Inst. Biol. Mar. Univ. R. G. Norte 2: 19–31.

———. 1973. Fisioecologia do peixe voador, *Hirundichthys affinis* (Günther, 1866). No nordeste Brasileiro. Crescimento, Reproducao e indices de captura. Msc. Thesis, Departamento de Fisiologia Geral e Instituto de Biologia Marinha. 72 p.

Didden, N. and F. Schott. 1993. Eddies in the North Brazil current retroflection region observed by geosat altimetry. J. Geophys. Res. 98: 20121–20131.

Ekau, W. and B. Knoppers, eds. 1996. Sedimentation processes and productivity in the continental shelf waters off east and northeast Brazil. Joint oceanographic projects, JOPS-II, Cruise report and first results. Centre for Tropical Marine Ecology, Bremen. 151 p.

Emery, A. R. 1972. Eddy formation from an oceanic island: Ecological effects. Carib. J. Sci. 12: 121–128.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) ver. 3.5c. Univ. Washington, Seattle, Washington.

Ferris, S. D. and W. J. Berg. 1987. The utility of mitochondrial DNA in fish genetics and fishery management. Pages 277–277 *in* F. Ryman and F. Utter, eds. Population genetics and fishery management. Univ. Washington Press, Seattle. 420 p.

Finnerty, J. R. and B. A. Block. 1992. Direct sequencing of mitochondrial DNA detects highly divergent haplotypes in blue marlin (*Makaira nigricans*). Mol. Mar. Biol. Biotech. 1: 206–214.

Fischer, W. ed. 1978. FAO species identification sheets for fishery purposes. Western Central Atlantic (fishing area 31). vols. 1–7: pag. var.

Frantatoni, D. M., W. E. Johns and T. L. Townsend. 1995. Rings of the North Brazil Current. Their structure and behavior inferred from observations and a numerical simulation. J. Geophys. Res. 100: 10633–10654.

Froelich, P. N. Jr., D. K. Atwood and G.S. Giese. 1978. Influence of Amazon River discharge on surface salinity and dissolved silicate concentration in the Caribbean Sea. Deep Sea Res. 25: 735–744.

Gable, F. J. 1993. Marine habitats: selected environmental and ecological charts. Pages 217–261 *in* G. A. Maul, ed. Climate change in the Intra-Americas Sea. E. Arnold, London.

Graves, J. E. and A. E. Dizon. 1989. Mitochondrial DNA sequence similarity of Atlantic and Pacific albacore tuna (*Thunnus alalunga*). Can. J. Fish. Aquat. Sci. 46: 870–873.

———, and J. R. McDowell. 1994. Genetic analysis of billfish population structure. ICCAT Collective volume of scientific papers, vol. 61: 505–515.

———, S. Ferris and A. E. Dizon. 1984. Close genetic similarity of Atlantic and Pacific skipjack tuna

(*Katsuwonus pelamis*) demonstrated with restriction endonuclease analysis of mitochondrial DNA. Mar. Biol. 79: 315–319.

Hateley, J.G. and T.D. Sleeter. 1993. A biochemical genetic investigation of spiny lobster (*Panulirus argus*) stock replenishment in Bermuda. Bull. Mar. Sci. 52: 993–1006.

Hunt von Herbing, I. and W. Hunte. 1991. Spawning and recruitment in the bluehead wrasse *Thalassoma bifasciatum* in Barbados. Mar. Ecol. Prog. Ser. 73: 28–41.

Hunte, W. 1986. Summary of the available database on oceanic pelagic fisheries in the Lesser Antilles. Pages 125–176 *in* R. Mahon, ed. Report and proceedings of the expert consultation on shared fishery resources of the Lesser Antilles region. Mayaguez, Puerto Rico, 8–12 September 1986. FAO Fish. Rpt. 383.

———, H. A. Oxenford and R. Mahon. 1995. Distribution and relative abundance of flyingfish (Exocoetidae) in the eastern Caribbean. II. Spawning substrata, eggs and larvae. Mar. Ecol. Prog. Ser. 117: 25–37.

Hynes, R. E., E. J. Duke and P. Joyce. 1989. Mitochondrial DNA as a genetic marker for brown trout, *Salmo trutta* L., populations. J. Fish. Biol. 35: 687–701.

Johns, W. E., T. N. Lee, F. A. Schott, R. J. Zantropp and R. H. Evans. 1990. The North Brazil Current retroflection: seasonal structure and eddy variability. J. Geophys. Res. 95: 22103–22120.

Khokiattiwong, S. 1988. Seasonal abundance and reproduction of the flyingfish, *Hirundichthys affinis* and *Parexocoetus brachypterus* near Barbados. M.Sc. Thesis, McGill Univ., Montreal, Canada. 152 p.

Kinder, T. 1983. Shallow currents in the Caribbean Sea and Gulf of Mexico as observed with satellite-tracked drifters. Bull. Mar. Sci. 33: 239–246.

Kjerfve, B. 1986. Physical flow processes in Caribbean waters over a range of scales. Pages 38–47 *in* J. C. Ogden and E. H. Gladfelter, eds. Caribbean coastal marine productivity. UNESCO Reports in Marine Science 41. 59 p.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Paabo, F. X. Villablanca and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Nat'l. Acad. Sci. U.S.A 86: 6196–6200.

Kotoulas, G., A. Magoulas, N. Tsimenides and E. Zouros. 1995. Marked mitochondrial DNA differences between Mediterranean and Atlantic populations of the swordfish, *Xiphias gladius*. Mol. Ecol. 4: 473–481.

Lacson, J. M. 1992. Minimal genetic variation among samples of six species of coral reef fishes collected at La Parguera, Puerto Rico, and Discovery Bay, Jamaica. Mar. Biol. 112: 327–331.

Lao, M. R. T. 1989. Distribution and abundance of flotsam, larval fish and juvenile fish off Barbados, with particular reference to the Exocoetidae. M.Sc. Thesis, McGill Univ., Montreal, Canada. 147 p.

Lawrence, H. N. 1993. The flyingfish fishery of Dominica. Page 159 *in* H.A. Oxenford, R. Mahon and W. Hunte, eds. The eastern Caribbean flyingfish project. OECS Fish. Rpt. no. 9. 171 p.

Lewis, J. B. 1964. Tagging experiments on the flyingfish *Hirundichthys affinis* (Günther). Bull. Mar. Sci. Gulf Carib. 14: 381–386.

Mahon, R., H. A. Oxenford and W. Hunte, eds. 1986. Development strategies for flyingfish fisheries of the eastern Caribbean. International Development Research Centre Man. Rpt. 128e. 148 p.

Martin, A. P., G. J. P. Naylor and S. Palumbi. 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. Nature 357: 153–155.

McElroy, D., P. Moran, E. Bermingham and I. Kornfield. 1992. REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. J. Hered. 83: 157–158.

Milner, G. B., D. J. Teel, F. M. Utter and G. A. Winans. 1985. A genetic method of stock identification in mixed populations of Pacific salmon, *Oncorhynchus* spp. Mar. Fish. Rev. 47: 1–8.

Mitton, J. B., C. J. Berg Jr. and K. S. Orr. 1989. Population structure, larval dispersal, and gene flow in the queen conch, *Strombus gigas,* of the Caribbean. Biol. Bull. 177: 356–362.

Molinari, R. L., M. Spillane, I. Brooks. D. Atwood and C. Duckett. 1981. Surface currents in the Caribbean Sea as deduced from Lagrangian observations. J. Geophys. Res. 86: 6537–6542.

Monteiro, A, A. C. A. El-Deir, R. P. Lessa and C. T. Lira. 1996. Fisheries biological research on *Hirundichthys affinis* in Brazil: an historical and current review. Presented at CARICOM Fisheries Resource Assessment and Management Program (CFRAMP) sub-projects specification workshop on small coastal pelagics and flyingfish. Grand Anse, Grenada, Sept. 11–13. SSW/WP/21.

Muller-Karger, F. E., C. R. McClain and P. L. Richardson. 1988. The dispersal of the Amazon's water. Nature 333: 57–59.

———, ———, T. R. Fisher. W. E. Esaias and R. Varela. 1989. Pigment distribution in the Caribbean Sea: observations from space. Prog. Oceanog. 23: 23–64.

Mulloney, B. C. 1961. A preliminary report on the results of tagging experiments on the flyingfish *Hirundichthys affinis* (Günther). West Indies Fish. Bull. 4: 1–4.

Nakashima, B. 1996. Report of the scoping mission for the small coastal pelagics and flyingfish subprojects. Presented at CARICOM fisheries resource assessment and management program

(CFRAMP) sub-projects specification workshop on small coastal pelagics and flyingfish. Grand Anse, Grenada, Sept. 11–13. SSW/WP/15.

Nei, M. 1987. Molecular evolutionary genetics. Colombia Univ. Press, New York. 512 p.

——— and W.-H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Nat'l. Acad. Sci. U.S.A. 76: 5269–5273.

——— and F. Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97: 207–217.

Ovenden, J. R. 1990. Mitochondrial DNA and marine stock assessment. A Review. Aust. J. Mar. Freshw. Res. 41: 835–853.

Oxenford, M. A. 1986. Synopsis of the biological data on the four-winged flyingfish, *Hirundichthys affinis* Günther. Pages 48–51 *in* R. Mahon, H. A. Oxenford and W. Hunte, eds. Development strategies for flyingfish fisheries of the eastern Caribbean. International Development Research Centre Man. Rpt. 128e.

———. 1991. Management of marine resources for sustainable development in the Caribbean. Pages 120–126 *in* E. A. Moore and J. Rudder, eds. Sustainable development for the Caribbean. Report on the CIDA\UWI Institutional Strengthening Project-funded Workshop on Sustainable Development, Univ. West Indies, Cave Hill Campus, Barbados, May 20–22, 1991.

———. 1994. Movements of flyingfish (*Hirundichthys affinis*) in the eastern Caribbean. Bull. Mar. Sci. 54: 49–62.

———, R. Mahon and W. Hunte, eds. 1993. The eastern Caribbean flyingfish project. OECS Fish. Rpt. no. 9. 171 p.

———, ———, and ———. 1995. Distribution and relative abundance of flyingfish (Exocoetidae) in the eastern Caribbean. 1. Adults. Mar. Ecol. Prog. Ser. 117: 11–23.

Palumbi, S., A. Martin, S. Romano, W. O. McMillan, L. Stice and G. Grabowski. 1991. The simple fool's guide to PCR. Dept. Zoology and Kewalo Marine Laboratory, Univ. Hawaii, Honolulu. 47 p.

Petersen, R. G. and L. Stramma. 1991. Upper level circulation in the South Atlantic Ocean. Prog. Oceanogr. 26: 1–73.

Roff, D. A. and P. Bentzen. 1989. The statistical analysis of mitochondrial polymorphisms: chi-square and the probability of small samples. Mol. Biol. Evol. 6: 539–545.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406–425.

Samlalsingh, S., E. Pandohee and E. Caesar. 1993. The Flyingfish fishery of Trinidad and Tobago. Page 160 *in* H. A. Oxenford, R. Mahon and W. Hunte, eds. The eastern Caribbean flyingfish project. OECS Fish. Rpt. no. 9. 171 p.

Schultz, E. T. and R. K. Cowen. 1994. Recruitment of coral-reef fishes to Bermuda: local retention or long-distance transport? Mar. Ecol. Prog. Ser. 109: 15–28.

Shulman, M. J. and E. Bermingham. 1995. Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution 49: 897–910.

Storey, K. W. 1983. Aspects of the biology and fishery of the flyingfish, *Hirundichthys affinis,* at Barbados. M. Phil. Thesis, Univ. West Indies, Barbados. 161 p.

Tringali, M. D. and R. R. Wilson Jr. 1993. Differences in haplotype frequencies of mtDNA of the Spanish sardine, *Sardinella aurita,* between specimens from the eastern Gulf of Mexico and southern Brazil. Fish. Bull., U.S. 91: 362–370.

Ward, R. D., N. G. Elliot, P. M. Grewe and A. J. Smolenski. 1994. Allozyme and mitochondrial DNA variation in yellowfin tuna from the Pacific Ocean. Mar. Biol. 118: 531–539.

Willoughby, S. 1993. The flyingfish fishery of Barbados. Page 159 *in* H. A. Oxenford, R. Mahon and W. Hunte, eds. The eastern Caribbean flyingfish project. OECS Fish. Rpt. no. 9. 171 p.

Zaneveld, J. S. 1961. The fishery resources and the fishery industries of the Netherlands Antilles. Proc. Gulf Carib. Fish. Inst. 14: 137–171.

Zwanenburg, K. C. T., P. Bentzen and J. M. Wright. 1992. Mitochondrial DNA differentiation in western north Atlantic populations of haddock (*Melanogrammus aeglefinus*). Can. J. Fish. Aquat. Sci. 49: 1959–1965.

DATE SUBMITTED: November 23, 1996. DATE ACCEPTED: April 18, 1997.

ADDRESSES: (C.G., H.A.O.) *Marine Resource and Environmental Management Programme (MAREMP), University of the West Indies, Cave Hill, Barbados;* (C.G., R.B.G.D.) *Department of Biological and Chemical Sciences, University of the West Indies, Cave Hill, Barbados.*